Catenands Built on Poly(ethylenimine). Attachment of Two Phenanthrolines in Close Proximity on the Polymer Backbone

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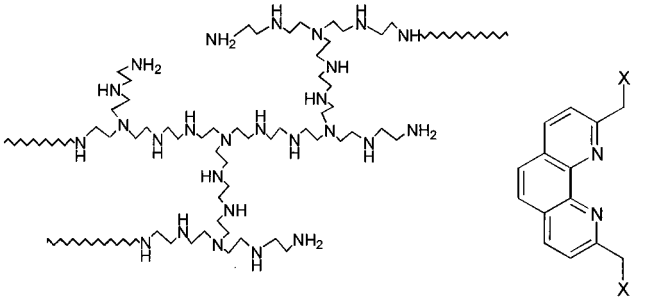
In an attempt to establish a methodology for introduction of two or more functional groups in close proximity to the backbone of synthetic polymers, two molecules of a 1,10-phenanthroline were preassembled with Cu(I) ion and then cross-linked with poly(ethylenimine) (PEI) to obtain $[Cu(I)Phen_2]^{PA}$PEI. Removal of Cu(I) ion from $[Cu(I)Phen_2]^{PA}$PEI produced $[apoPhen_2]^{PA}$PEI, whose primary and secondary amines were subsequently acetylated to produce $[apoPhen_2]^{PA}$AcPEI. Cross-linkage of each preassembled phenanthroline pair with PEI through four-point attachment would produce two interlocking macrocycles linked to each other or two fused entwining macrocycles. The former is a special class of catenand, whereas the latter is a catenand analogue. Ionization constants for the phenanthroline moieties and formation constants for the Cu(II) complex were measured for the PEI derivatives. The results indicate that the geometry of two phenanthrolines originally assembled by Cu(I) ion is effectively conserved in $[apoPhen_2]^{PA}$PEI and $[apoPhen_2]^{PA}$AcPEI. Stabilization of a phenanthrolinium ion by an adjacent phenanthroline as well as unfavorable electrostatic interaction between two close phenanthrolinium ions are disclosed by the $10^{9.5}$-fold difference in ionization constants for two preassembled phenanthrolines. Analysis of the Cu(II)-binding data revealed that the effective molarity of a phenanthroline toward Cu(II) ion bound to the other phenanthroline in each phenanthroline pair is 10^6 M. These demonstrate very close proximity between the two phenanthrolines. Preassemblage of organic molecules with a template metal ion followed by cross-linkage with a branched polymer can be utilized in design of biomimetic functional molecules including artificial enzymes.

For activities of biotic functional molecules in processes such as catalysis, antigen binding, signal transduction, and electron or energy transport, positioning of two or more functional groups in optimum locations is essential. This has been, therefore, among the basic objectives in many studies to design effective biomimetic molecules.

In the area of molecular recognition,[1] for example, various efforts have been made to introduce multiple interaction sites in host molecules to facilitate selective and strong complexation with guest molecules. For designing catalytic antibodies,[2] induction of binding and catalytic groups in the productive positions is one of the main targets. In devising artificial enzymes based on synthetic molecules,[3] functional groups exploited by enzymes must be incorporated in the artificial active site and the convergent catalytic groups must be finely aligned to achieve cooperative catalytic participation in the chemical transformation of the bound substrate.

Active sites that can accommodate finely aligned multiple catalytic groups would not be built easily with molecular skeletons provided by relatively small synthetic hosts. For this reason, branched poly(ethylenimine) (PEI; MW ca. 60 000) has been exploited as a molecular backbone for artificial enzymes.[4] PEI contains ethylamine as the repeating unit. Among ca. 1400 amino groups of PEI, ca. 350 are primary amines, ca. 700 are secondary amines, and ca. 350 are tertiary amines. The tertiary amines are the branching points on the macromolecular backbone, and PEI is highly branched and water-soluble.



PEI

A: X = Br

B: X = H

In an attempt to establish a methodology for introduction of two or more groups in close proximity in the artificial active sites built on polymeric backbones, PEI has been employed as a macromolecular spacer equipped with a large number of nucleophilic sites.[5] In this study,

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we carried out cross-linkage with PEI of two molecules of 2,9-bis(bromomethyl)-1,10-phenanthroline (A) preassembled by Cu(I) ion. This may be related to linkage of 2,9-diaryl-1,10-phenanthrolines coordinated to a metal template such as Cu(I), which has been used in preparation of many catenands.[6] In this paper, formation of proximal pairs of phenanthroline on the PEI backbone after removal of the template Cu(I) ion will be described. In addition, the close proximity between the two phenanthrolines will be demonstrated by remarkable stabilization of phenanthrolinium cations by the adjacent phenanthroline moieties as well as extraordinary effective molarity of the two phenanthrolines in binding of a Cu(II) ion.

Experimental Section

Cross-Linkage of Cu(I)A_2 with PEI. PEI (MW 50 000−60 000, purchased from Aldrich) was purified by dialysis (cutoff MW 12 000). A solution of CuI (0.22 g, 1.2 mmol) and A (0.85 g, 2.3 mmol) dissolved in 80 mL of dimethyl sulfoxide (DMSO) and 30 mL of methanol was added dropwise to a solution of PEI (5.0 g, 0.12 residue mol) dissolved in 70 mL of DMSO and 40 mL of methanol over a period of 30 min at 25 °C. After the mixture was left at 40 °C for 2 days, the product was purified by dialysis against 30% (v/v) aqueous ethanol (five times), 10% (v/v) aqueous ethanol (three times), 0.1 M NaCl solution (five times), and water (five times). On the basis of the amount of Cu incorporated into the product determined by ICP analysis, the content of the bisphenanthroline complex of Cu(I) ion in the product, designated as [Cu(I)$Phen_2$]$_{0.87}^{PA}$PEI, was estimated as 0.87 residue mol % of PEI. By separate preparations, [Cu(I)$Phen_2$]$_{0.56}^{PA}$PEI and [Cu(I)$Phen_2$]$_{3.2}^{PA}$PEI were obtained. For these PEI derivatives, the content of the bisphenanthroline complex of Cu(I) ion was estimated as 0.56 residue mol % and 3.2 residue mol %, respectively, by ICP analysis. The content of phenanthroline moiety in [Cu(I)-$Phen_2$]$_{0.56}^{PA}$PEI was estimated as 1.2 residue mol % by NMR analysis using 1,3,5-benzenetricarboxylate as the internal standard. This agrees well with the Cu(I) content estimated by ICP analysis.

Demetalation of [Cu(I)$Phen_2$]^{PA}PEI. Samples of [Cu(I)-$Phen_2$]^{PA}PEI were dialyzed against 1% NaCN solution (three times), 0.1 M NaCl (five times), and deionized water (five times). ICP measurement indicated that more than 99% of Cu(I) ion was removed in the demetalated PEI derivatives (designated as [apo$Phen_2$]$_{0.56}^{PA}$PEI and [apo$Phen_2$]$_{3.2}^{PA}$PEI, depending on the Cu(I) content of the original PEI derivative).

Acetylation of [apo$Phen_2$]$_{0.56}^{PA}$PEI. By dropwise addition of acetic anhydride (20 mL) to [apo$Phen_2$]$_{0.56}^{PA}$PEI (0.73 mM in terms of Cu(I)-binding site, 500 mL) at pH 7−8 and 25 °C, [apo$Phen_2$]$_{0.56}^{PA}$PEI was acetylated to produce [apo-$Phen_2$]$_{0.56}^{PA}$AcPEI, which was purified by dialysis. Treatment of PEI derivatives with excess acetic anhydride has been shown to effectively acetylate primary and secondary amino groups of PEI.[7]

Random Attachment of A to PEI. After incubation at 45 °C for 2 days, the mixture of PEI (4.6 g, 0.11 residue mol) and A (0.84 g, 2.3 mmol) in 230 mL of DMSO was dialyzed as described above for [Cu(I)$Phen_2$]$_{0.87}^{PA}$PEI, leading to a product (designated as [Phen]$_{0.81}^{Ran}$PEI) in which phenanthroline moieties are randomly attached to PEI. The content of the phenanthroline moiety was estimated as 0.81% of PEI monomer residues by NMR spectrometry using 1,3,5-benzenetricarboxylate as the internal standard.

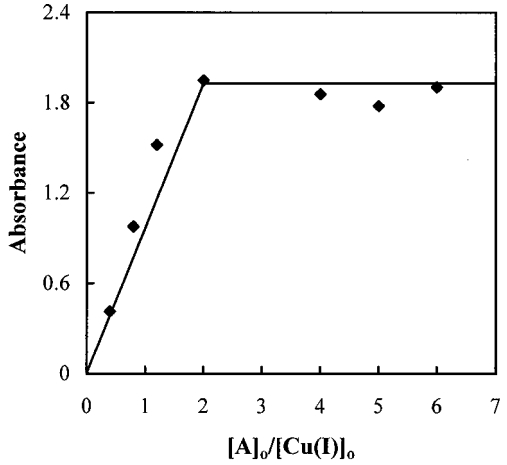

Figure 1. Absorbance change observed at 468 nm as the ratio of the initially added concentration of A ($[A]_o$) and that of Cu(I) ($[Cu(I)]_o$ was varied at 25 °C in DMSO−methanol (7:4 (v/v)). $[Cu(I)]_o$ was kept at 4.84×10^{-4} M.

Acetylation of [Phen]$_{0.81}^{Ran}$PEI. [Phen]$_{0.81}^{Ran}$PEI was acetylated with acetic anhydride and purified by dialysis to obtain [Phen]$_{0.81}^{Ran}$AcPEI as described above for acetylation of [apo$Phen_2$]$_{0.56}^{PA}$PEI.

Measurements. Spectrophotometric measurements were performed with a Hewlett Packard 8453 UV−vis spectrophotometer. Temperature was controlled with a Fisher Scientific Isotherm circulator model 70. pH was measured with a Dongwoo Medical System (DP880) pH/ion meter. Below pH 2 and above pH 12, pH was calculated from $[H^+]$ or $[OH^-]$. For spectral titration, pH of the buffer solution was changed by addition of NaOH or HCl solution, taking advantage of the buffer capacity[8] of PEI derivatives at pH 2−12. For metal-binding studies, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (pH 7−8), boric acid (pH 9−10), sodium carbonate (pH 11), and sodium phosphate (pH 12) were used as buffers (0.05 M), and buffer solutions contained 0.1 M NaCl. ICP analysis of metal ions was carried out with a Perkin-Elmer Plasma 40.

Results

The absorbance change observed upon addition of various concentrations of A to a solution of CuI in a DMSO−methanol mixture is illustrated in Figure 1. The data indicate that Cu(I) and A produce Cu(I)A_2 exclusively when they are mixed in a 1:2 molar ratio. This agrees with formation of distorted-tetrahedral Cu(I) complexes reported for 2,9-dialkyl-1,10-phenanthrolines.[9]

By the reaction of PEI with Cu(I)A_2 in a DMSO−methanol mixture, two molecules of A preassembled by Cu(I) ion were cross-linked with PEI to obtain [Cu-(I)$Phen_2$]$_{0.56}^{PA}$PEI, [Cu(I)$Phen_2$]$_{0.87}^{PA}$PEI, and [Cu(I)-$Phen_2$]$_{3.2}^{PA}$PEI, which were purified by repetitive dialysis. The Cu(I) contents of [Cu(I)$Phen_2$]$_{0.56}^{PA}$PEI, [Cu(I)-$Phen_2$]$_{0.87}^{PA}$PEI, and [Cu(I)$Phen_2$]$_{3.2}^{PA}$PEI were estimated as 0.56, 0.87, and 3.2 residue mol %, respectively, by ICP analysis. In the case of [Cu(I)$Phen_2$]$_{0.56}^{PA}$PEI, the content of the phenanthroline moieties was estimated separately as 1.2 residue mol % by NMR spectrometry using 1,3,5-benzenetricarboxylate as the internal standard. This corresponds to the presence of 2.1 phenanthroline moi-

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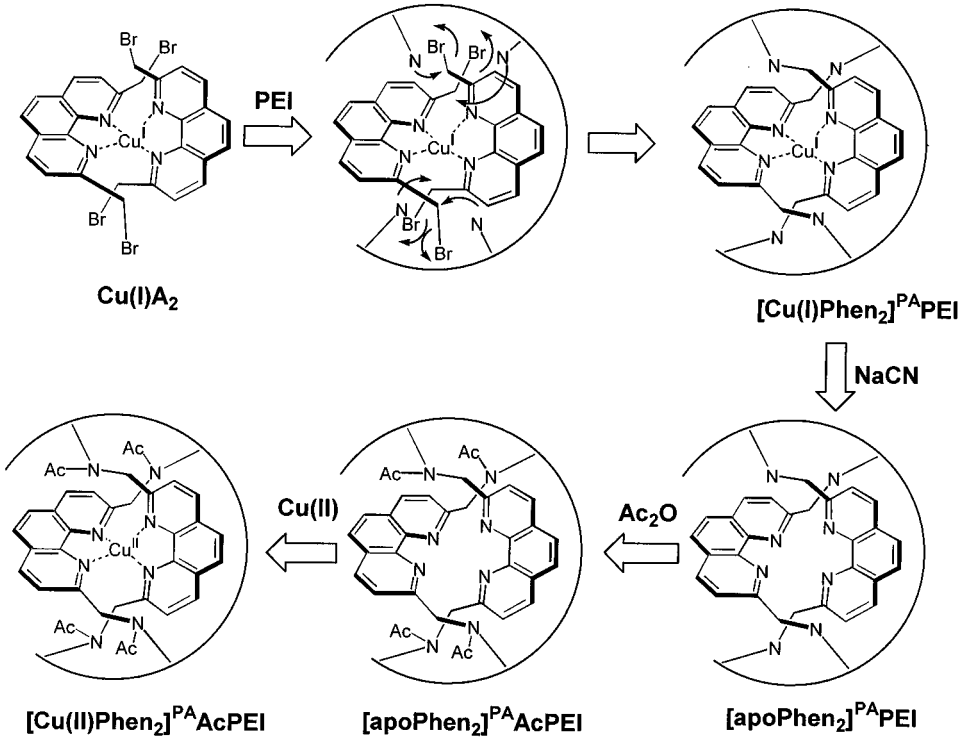
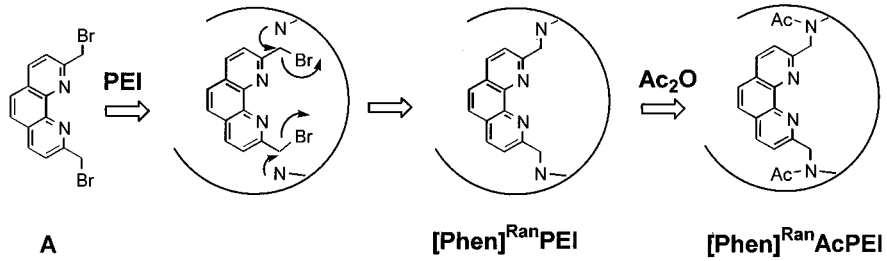
Scheme 1



Scheme 2



eties for each Cu(I) ion. By treatment with NaCN, Cu(I) ion was removed to produce $[apoPhen_2]_{0.56}{}^{PA}PEI$ and $[apoPhen_2]_{3.2}{}^{PA}PEI$. The primary and secondary amino groups of $[apoPhen_2]_{0.56}{}^{PA}PEI$ were acetylated by treatment with excess acetic anhydride to obtain $[apoPhen_2]_{0.56}{}^{PA}AcPEI$.

By the reaction of A with PEI in the absence of Cu(I) ion, phenanthroline moieties were attached randomly to PEI to obtain $[Phen]_{0.81}{}^{Ran}PEI$. $[Phen]_{0.81}{}^{Ran}PEI$ was converted to $[Phen]_{0.81}{}^{Ran}AcPEI$ by acetylation of the primary and the secondary amino groups.

Preparation of various PEI derivatives in which two preassembled phenanthroline moieties are cross-linked by the polymer backbone is schematically presented in Scheme 1. Synthesis of the PEI derivatives in which the phenanthroline moiety is randomly attached to the polymer backbone is schematically illustrated in Scheme 2. In Schemes 1 and 2, the amino groups attacking A may be either primary or secondary amines. Once the first bromomethyl group is attacked by an amino group of PEI, the subsequent reactions are expected to occur readily in view of the intramolecular nature of the reactions and availability of plenty of amino groups in the vicinity. Each molecule of $Cu(I)A_2$ cross-linked with PEI would lead to formation of two macrocycles.[10] On the other hand, each molecule of A randomly attached to PEI may be accompanied by formation of a macrocycle.[10] By acetylation with acetic anhydride, primary and secondary amino groups are converted into acetamino groups whereas tertiary amino groups remain unchanged.

The UV−vis spectra of the PEI derivatives containing phenanthrolines measured at various pH values provide information on the protonation of the phenanthroline moieties. This is because phenanthroline is the only strong chromophore available on the polymers. The UV−vis spectra of $[apoPhen_2]_{0.56}{}^{PA}PEI$, $[apoPhen_2]_{0.56}{}^{PA}AcPEI$, $[Phen]_{0.81}{}^{Ran}PEI$, and $[Phen]_{0.81}{}^{Ran}AcPEI$ were obtained at pH 0−14 (see the Supporting Information). Examination of pH dependence of absorbance at various wavelengths revealed that the spectral titration data agree with ionization of diacidic species in the case of $[apoPhen_2]_{0.56}{}^{PA}PEI$ and $[apoPhen_2]_{0.56}{}^{PA}AcPEI$ as illustrated in Figures 2 and 3. On the other hand, the spectral titration data agree with ionization of monoacidic species in the case

(10) Although direct evidence has not been obtained to prove that all of the four bromomethyl groups of $Cu(I)A_2$ are attacked by the amino groups of one molecule of PEI during the cross-linkage, the close proximity between the paired phenanthrolines in the resulting PEI derivative strongly suggests that the cross-linkage occurs through four-point attachment to one PEI. Even if the phenanthroline is linked to PEI by a single attachment in $[Phen]_{0.81}{}^{Ran}PEI$, the conclusions of this study are not affected significantly.

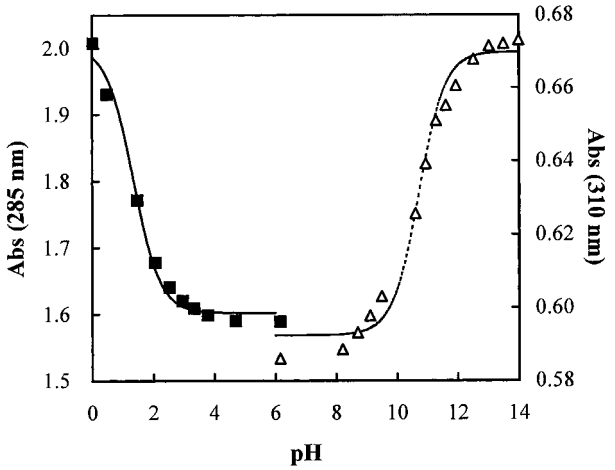


Figure 2. Spectral titration of $[apoPhen_2]_{0.56}{}^{PA}PEI$ measured at 285 nm (■) or 310 nm (△) and 25 °C. The concentration of phenanthroline moiety in $[apoPhen_2]_{0.56}{}^{PA}PEI$ was 7.91×10^{-5} M.

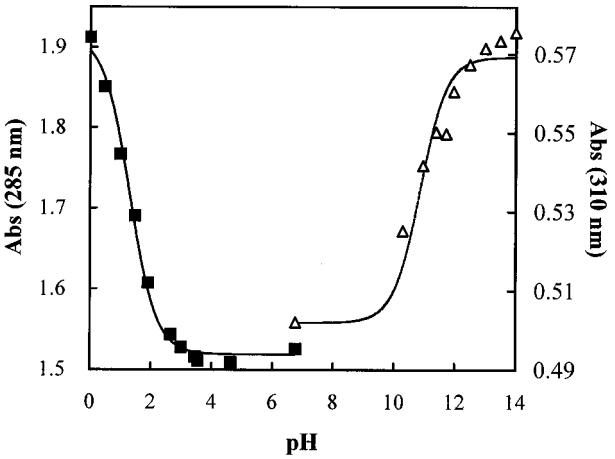


Figure 3. Spectral titration of $[apoPhen_2]_{0.56}{}^{PA}AcPEI$ measured at 285 nm (■) or 310 nm (△) and 25 °C. The concentration of phenanthroline moiety in $[apoPhen_2]_{0.56}{}^{PA}AcPEI$ was 7.91×10^{-5} M.

of $[Phen]_{0.81}{}^{Ran}PEI$ and $[Phen]_{0.81}{}^{Ran}AcPEI$ as illustrated in Figures 4 and 5. By analysis with a nonlinear regression program, pK_a values were obtained from the data of Figure 2−5 and are summarized in Table 1.

To examine ability of the $[apoPhen_2]^{PA}PEI$ derivatives to recognize metal ions, formation constants (K_f) for the Cu(II) complexes of $[apoPhen_2]_{0.56}{}^{PA}AcPEI$ and $[Phen]_{0.81}{}^{Ran}$-AcPEI were measured.[11] Absorbance changes (Figure 6) observed upon addition of Cu(II) ion to $[apoPhen_2]_{0.56}{}^{PA}$-AcPEI and $[Phen]_{0.81}{}^{Ran}AcPEI$ revealed that Cu(II) binding involves coordination of phenanthrolines and that the amounts of Cu(II) ion strongly bound to $[apoPhen_2]_{0.56}{}^{PA}$-AcPEI and $[Phen]_{0.81}{}^{Ran}AcPEI$ were 0.52 and 0.79 residue mol %, respectively. This indicates that binding of one

(11) Binding of Cu(II) ion to $[apoPhen_2]_{0.56}{}^{PA}PEI$ or $[Phen]_{0.81}{}^{Ran}PEI$ was not investigated due to the complications arising from Cu(II) binding[12] by the ethylenediamine moieties of the PEI backbone. When the primary and secondary amino groups of PEI are acetylated as in $[apoPhen_2]_{0.56}{}^{PA}AcPEI$ or $[Phen]_{0.81}{}^{Ran}AcPEI$, metal binding by the polymer backbone alone can be neglected.

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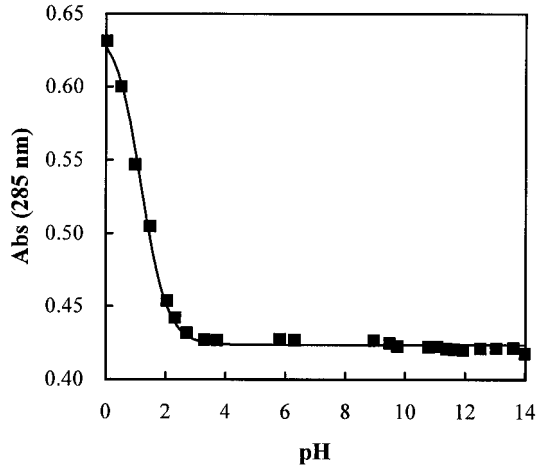


Figure 4. Spectral titration of $[Phen]_{0.81}{}^{Ran}PEI$ measured at 285 nm and 25 °C. The concentration of phenanthroline moiety in $[Phen]_{0.81}{}^{Ran}PEI$ was 3.73×10^{-5} M.

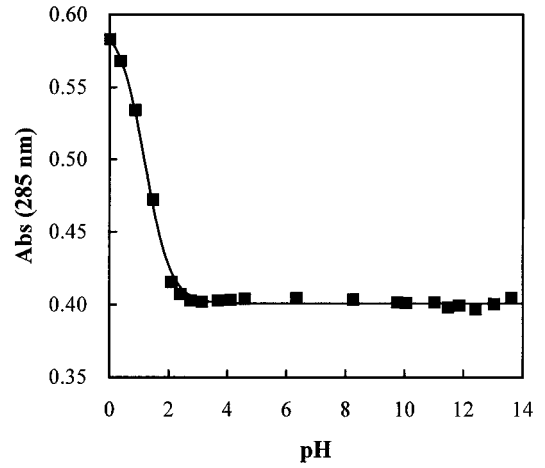


Figure 5. Spectral titration of $[Phen]_{0.81}{}^{Ran}AcPEI$ measured at 285 nm and 25 °C. The concentration of phenanthroline moiety in $[Phen]_{0.81}{}^{Ran}AcPEI$ was 3.73×10^{-5} M.

Table 1. pK_a Values for Various PEI Derivatives at 25 °C Estimated by Analysis of Spectral Titration Data Illustrated in Figures 2−5

polymer	parameter	value
$[apoPhen_2]_{0.56}{}^{PA}PEI$	pK_{a1}	1.34 ± 0.08
	pK_{a2}	10.77 ± 0.07
$[apoPhen_2]_{0.56}{}^{PA}AcPEI$	pK_{a1}	1.30 ± 0.06
	pK_{a2}	10.87 ± 0.17
$[Phen]_{0.81}{}^{Ran}PEI$	pK_a	1.18 ± 0.04
$[Phen]_{0.81}{}^{Ran}AcPEI$	pK_a	1.20 ± 0.03

Cu(II) ion involves two phenanthroline moieties in the case of $[apoPhen_2]_{0.56}{}^{PA}AcPEI$ and one phenanthroline moiety in the case of $[Phen]_{0.81}{}^{Ran}AcPEI$.

Various types of small molecules such as metal ions and organic molecules are complexed to PEI derivatives. Although each molecule of a PEI derivative can bind many small molecules, binding of the small molecules can be simplified as independent complexation to individual binding sites. This approximation is valid as far as the complexation to a binding site does not affect the succeeding bindings and has been found to be valid for binding of several types of small molecules to PEI derivatives.[4c−e,5b,13] The number of Cu(II) binding sites in $[apoPhen_2]_{0.56}{}^{PA}AcPEI$ or $[Phen]_{0.81}{}^{Ran}AcPEI$ is 7.8 or 11, respectively, for PEI with 1400 monomer residues.

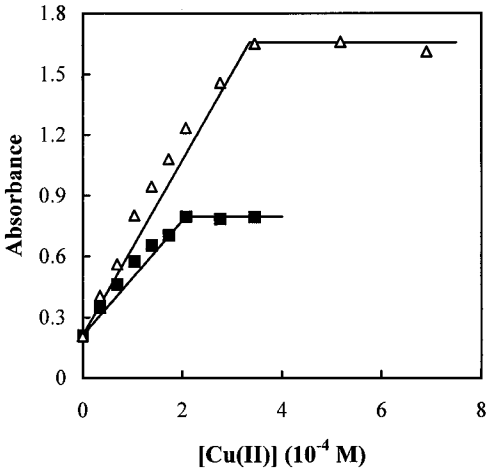


Figure 6. Absorbance change observed at 440 nm for [apoPhen$_2$]^{PA}AcPEI (■) and [Phen]^{Ran}AcPEI (△) at pH 7.0 and 25 °C in the presence of various amounts of Cu(II)Cl$_2$. The concentration of phenanthroline moiety in [apoPhen$_2$]$_{0.56}^{PA}$AcPEI was 4.82×10^{-4} M and that in [Phen]$_{0.81}^{Ran}$AcPEI was 3.55×10^{-4} M.

Although the structures and metal-binding abilities of the Cu(II) binding sites may not be identical, it is reasonable to assume that the Cu(II)-binding to one site does not affect succeeding processes.

Since the binding of Cu(II) ion by [apoPhen$_2$]$_{0.56}^{PA}$AcPEI and [Phen]$_{0.81}^{Ran}$AcPEI was very strong, the K_f values were estimated by measuring the equilibrium constant (K_{ex}) for extraction of the bound metal ion from [Cu(II)-Phen$_2$]$_{0.56}^{PA}$AcPEI or [Cu(II)Phen]$_{0.81}^{Ran}$AcPEI with a competing ligand (CL)[5b,13] according to the scheme of eqs 1−3. From this, eqs 4 and 5 are derived for dependence

$$\text{Cu(II)-BS-PEI} + 2\text{CL} \overset{K_{ex}}{\rightleftharpoons} \text{Cu(II)(CL)}_2 + \text{BS-PEI} \quad (1)$$

$$\text{Cu(II)} + \text{BS-PEI} \overset{K_f}{\rightleftharpoons} \text{Cu(II)-BS-PEI} \quad (2)$$

$$\text{Cu(II)} + 2\text{CL} \overset{K_f^{CL}}{\rightleftharpoons} \text{Cu(II)(CL)}_2 \quad (3)$$

$$\text{Abs} = (\epsilon_{\text{Cu(II)-BS-PEI}} - \epsilon_{\text{BS-PEI}})[\alpha - (\alpha^2 - 4[\text{Cu(II)}]_o[\text{BS} - \text{PEI}]_o)^{1/2}]/2 + \epsilon_{\text{BS-PEI}}[\text{BS-PEI}]_o \quad (4)$$

$$\alpha = K_{ex}[\text{CL}]_o^2 + [\text{BS-PEI}]_o + [\text{Cu(II)}]_o \quad (5)$$

of absorbance (Abs) of the equilibrium mixture on the initially added concentration ([CL]$_o$) of CL in the presence of excess CL. Here, BS stands for metal-binding site unoccupied by the metal ion. The equations are based on formation of a 1:2-type complex, Cu(II)(CL)$_2$, between Cu(II) and CL. When the CL forms a 1:1-type complex, Cu(II)(Cl), the equations were modified accordingly. In the equations, ϵ terms represent molar extinction coefficients and the concentration terms stand for the initially added concentrations.

The degree of exchange of Cu(II) ion between the phenantrholine-containing PEI and CL can be measured

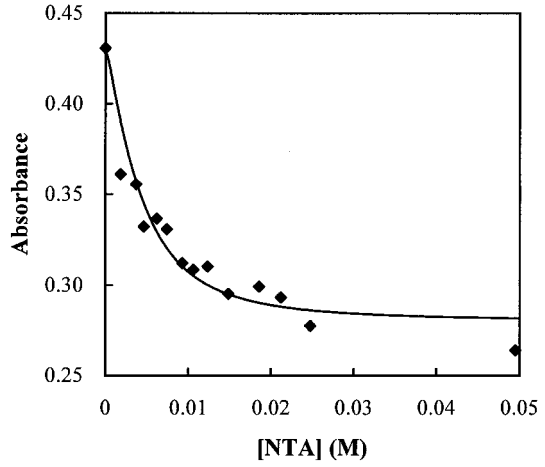


Figure 7. Absorbance change observed at 440 nm when NTA was added to a solution of [apoPhen$_2$]$_{0.56}^{PA}$AcPEI (1.68×10^{-4} M in terms of Cu(II) binding site) and Cu(II)Cl$_2$ (1.44×10^{-4} M) at pH 12.0 and 25 °C. The theoretical line was obtained by analysis according to eqs 4 and 5.

Table 2. Values of log pK_f Measured for [Cu(II)Phen$_2$]$_{0.56}^{PA}$PEI and [Cu(II)Phen]$_{0.81}^{Ran}$AcPEI at 25 °C

complex[a]	pH	log K_f	competing ligand[b]
[Cu(II)Phen$_2$]$_{0.56}^{PA}$AcPEI	7.00	12.11 ± 0.13	NTA
	8.00	14.02 ± 0.12	NTA
	9.00	15.53 ± 0.09	NTA
	10.00	16.36 ± 0.09	NTA
	11.00	17.05 ± 0.10	NTA
	12.00	16.62 ± 0.17	NTA
[Cu(II)Phen]$_{0.81}^{Ran}$AcPEI	8.00	13.47 ± 0.09	NDPA
	9.00	13.39 ± 0.07	NDPA
	10.00	13.81 ± 0.05	NDPA

[a] Cu(II) complexes of [apoPhen$_2$]$_{0.56}^{PA}$AcPEI and [Phen]$_{0.81}^{Ran}$AcPEI were prepared by addition of 0.8−0.9 equiv of CuCl$_2$ to the solutions of respective PEI derivatives. [b] With Cu(II) ion, NTA (nitrilotriacetic acid) forms Cu(II)(NTA)$_2$ and NDPA (nitrilodiacetic-3-propionic acid) forms Cu(II)(NDPA).

spectrophotometrically. An example of the raw data obtained for measurement of K_f at a fixed pH is illustrated in Figure 7. The curve of Figure 7 was obtained by analysis according to eqs 4 and 5 with a nonlinear regression program, and the value of K_f was calculated from K_{ex} (= K_f^{CL}/K_f) estimated therefrom and K_f^{CL} values reported[14] in the literature. The values of K_f thus obtained for [Cu(II)Phen$_2$]$_{0.56}^{PA}$AcPEI and [Cu(II)-Phen]$_{0.81}^{Ran}$AcPEI at various pH values are summarized in Table 2 and Figure 8. The K_f represents the average value of the formation constants for the individual Cu(II)-binding sites on the PEI derivatives.

The theoretical line drawn for [Cu(II)Phen$_2$]$_{0.56}^{PA}$AcPEI in Figure 8 is based on the assumption that a base with a pK_a of 10.87 (same as pK_{a2} listed in Table 1) binds Cu(II) ion with log K_f° (the limiting value of K_f) of 17.0 and protonation of the base blocks Cu(II) binding. The large deviation seen at pH 7 suggests that protonation of the PEI backbone exerts inhibitory effects on Cu(II) binding. The pH independence of log K_f for [Cu(II)-Phen]$_{0.81}^{Ran}$AcPEI indicates that the phenanthrolines are in a fully active form over the pH range examined. The

(13) (a) Suh, J.; Hong, S. H. *J. Polymer Sci. A.: Polym. Chem.* **1997**, *35*, 533. (b) Suh, J.; Lee, S. H.; Kim, S. M.; Hah, S. S. *Bioorg. Chem.* **1997**, *25*, 221. In this paper, K_{ex} should be replaced with $1/K_{ex}$ in Eqs 3−5 and the accompanying expressions for α. In the expressions for α, [CL]$_o^n$ (n = 1−3) should be replaced with −[CL]$_o^n$.

(14) Martell, A. E.; Smith, R. M. In *Critical Stability Constants*; Plenum: New York, 1974−1982; Vols. 1−5.

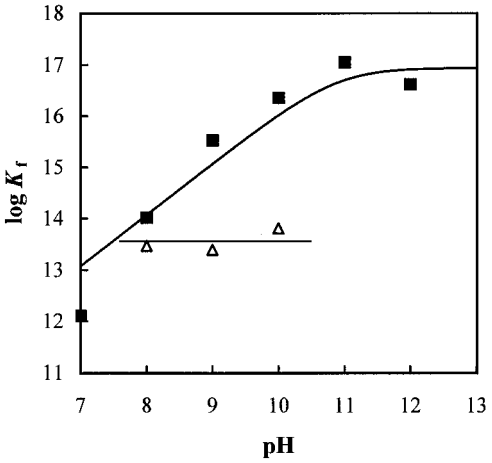
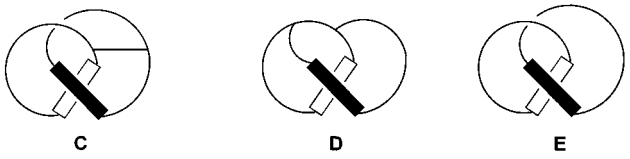


Figure 8. pH dependence of log K_f for $[apoPhen_2]_{0.56}^{PA}$AcPEI (■) and $[Phen]_{0.81}^{Ran}$AcPEI (△). See the text for construction of the theoretical lines.

line drawn for $[Cu(II)Phen]_{0.81}^{Ran}$AcPEI in Figure 8 represents the average value (13.6) of log K_f.

Discussion

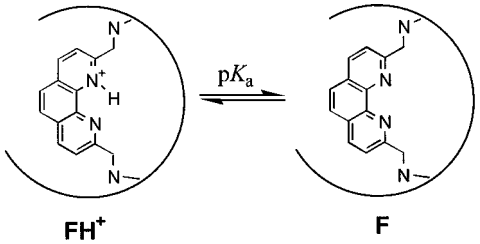
Interlocking macrocycles are called catenanes, whereas interlocking macrocycles with ligand properties and their complexes are named catenands and catenates, respectively.[6] Many catenands have been prepared by linkage of 2,9-diaryl-1,10-phenanthrolines coordinated to a metal template followed by removal of the metal ion from the resulting catenates.[6a,b,15] Cross-linkage of two preassembled phenanthrolines through four-point attachment (Scheme 1) is expected to lead to two interlocking macrocycles linked to each other (C) or two fused macrocycles (D), depending on the relative positions of the four amine nucleophiles on PEI backbone. The two



interlocking macrocycles of C represent a special class of catenand. Due to the steric constraints imposed by PEI, the two macrocycles of D would keep entwining conformation. In this regard, D can be considered as a catenand analogue. In C or D, conformational freedom of the two macrocycles is suppressed considerably more than that in separated interlocking macrocycles (E) of a conventional catenand, especially when C or D is built on highly branched PEI backbone.

The two phenanthrolines contained in a catenand or a catenand analogue would not remain in close proximity unless the conformational freedom of the two entwining macrocycles is sufficiently suppressed. Information on conformation of the phenanthroline pair in a catenate or the corresponding catenand may be obtained by NMR spectral analysis. For the catenates or catenands built on PEI, characterization of the phenanthroline as well as the methylene groups attached to positions 2 and 9 of the phenanthroline by ^{1}H NMR spectrometry (500 M Hz, D_2O) was unsuccessful due to the prevailing peaks of the protons present on the PEI backbone even after exchange of hydrogens of amino groups with deuteriums by treating with D_2O. PEI derivatives with a higher content of phenanthrolines, $[Cu(I)Phen_2]_{3.2}^{PA}$PEI and $[apoPhen_2]_{3.2}^{PA}$PEI, were prepared and used in the NMR study to raise the intensities of NMR peaks of the phenanthroline moiety.

In spite of the failure to obtain positive results by NMR analysis, that the two phenanthroline moieties of the two entwining macrocycles in $[apoPhen_2]_{0.56}^{PA}$PEI or $[apoPhen_2]_{0.56}^{PA}$AcPEI are positioned in close proximity is evidenced by the results of the spectral titration illustrated in Figures 2−5. For $[Phen]_{0.81}^{Ran}$PEI and $[Phen]_{0.81}^{Ran}$AcPEI, the phenanthrolinium moieties attached randomly to the polymer can be treated as monoacidic species (e.g., FH^+ and F: protonation[16] or acetylation of the amino groups of the polymer backbone is omitted to simplify illustration) with pK_a of 1.2 (Table 1). The phenanthroline moieties introduced to $[Phen]_{0.81}^{Ran}$PEI and $[Phen]_{0.81}^{Ran}$AcPEI would not affect ionization of one another appreciably. Ionization of the phenanthroline moieties should be affected by amino groups of the PEI backbone, which are protonated at low pH, as well as decreased polarity of the microdomains containing many acetamino groups. Both the ammonium cation and the decreased polarity are expected to suppress protonation of the phenanthroline moieties. This is reflected by much lower pK_a of $[Phen]_{0.81}^{Ran}$PEI and $[Phen]_{0.81}^{Ran}$AcPEI compared with that[14] (5.85) of B.[17] Similar pK_a values observed for $[Phen]_{0.81}^{Ran}$PEI and $[Phen]_{0.81}^{Ran}$AcPEI suggest that the greater cationic nature of $[Phen]_{0.81}^{Ran}$PEI and the more nonpolar microenvironments of $[Phen]_{0.81}^{Ran}$AcPEI exert comparable effects on lowering the pK_a value.



For $[apoPhen_2]_{0.56}^{PA}$PEI or $[apoPhen_2]_{0.56}^{PA}$AcPEI, the spectral titration (Figures 2 and 3) indicates two stages of ionization associated with pK_a of ca. 1.3 and ca. 10.8 (Table 1). Unlike phenanthrolines attached to $[Phen]_{0.81}^{Ran}$PEI or $[Phen]_{0.81}^{Ran}$AcPEI, the pair of phenanthrolines preassembled by Cu(I) and then cross-linked with PEI appears to behave as one unit in ionization. As illustrated by GH_2^{2+}, GH^+, and G (protonation[16] or acetylation of the amino groups of the polymer backbone is

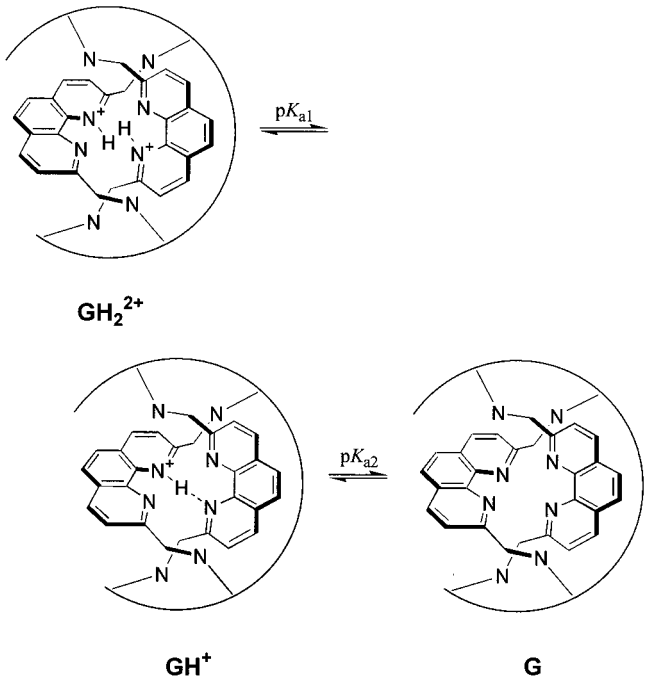
(15) (a) Cesario, M.; Dietrich, C. O.; Edel, A.; Guilhem, J.; Kintzinger, J.-P.; Pascard, C.; Sauvage, J.-P. *J. Am. Chem. Soc.* **1986**, *108*, 6250. (b) Dietrich-Buchecker, C. O.; Sauvage, J.-P.; Kern, J.-M. *J. Am. Chem. Soc.* **1989**, *111*, 7791. (c) Nierengrten, J.-F.; Dietrich-Buchecker, C. O.; Sauvage, J.-P. *J. Am. Chem. Soc.* **1994**, *116*, 375. (d) Amabilino, D. B.; Dietrich-Buchecker, C. O.; Livoreil, A.; Pérez-García, L.; Sauvage, J.-P.; Stoddart, J. F. *J. Am. Chem. Soc.* **1996** *118*, 3905.

(16) The amino group would be mostly protonated at pH <2 and mostly unprotonated at pH >12.[8]

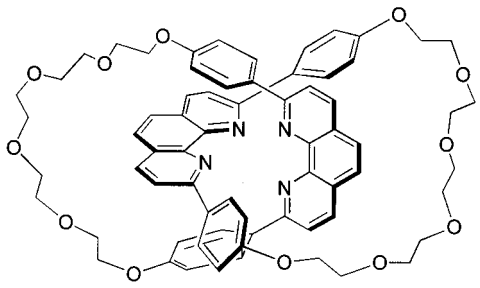
(17) The acetamino and amino groups attached to the methyl groups of B would not exert significant effect on the basicity or metal binding ability of phenanthroline nitrogens in view of the similar Hammett σ values of acetaminomethyl, aminomethyl, and methyl groups.[18]

(18) (a) Charton, M. *Prog. Phys. Org. Chem.* **1981**, *13*, 119. (b) Isaacs, N. In *Physical Organic Chemistry*, 2nd ed.; Longman: Essex, 1995; pp 152−153.

omitted to simplify illustration), the phenanthrolinium pair acts as a diacidic species.



The pK_{a2} of $[apoPhen_2]_{0.56}^{PA}$PEI or $[apoPhen_2]_{0.56}^{PA}$AcPEI is greater by ca. 9.5 pK_a units than the corresponding pK_{a1}. Thus, the phenanthrolinium of GH^+ is 3×10^9 times less acidic than that in GH_2^{2+}. This pK_a difference is attributable to destabilization of GH_2^{2+} by electrostatic interaction between two cations and stabilization of GH^+ by the hydrogen bond between two phenanthroline moieties. Both of these two factors originate from the close proximity between the two preassembled phenanthrolines. The degree of stabilization of GH^+ can be estimated by comparing the basicities of G and B. The pK_a of GH^+ is larger than that of the conjugate acid of B by 5 pK_a units. Considering the possible reduction of pK_a of GH^+ by hydrophobic microenvironments provided by paired phenanthrolines as well as acetamino groups in addition to that by nearby ammonium ions, the intrinsic basicity of G should be more than 10^5 times greater than that of B.



Sauvage and co-workers reported[15a] the crystal structure of the proton catenate of H, in which one of the nitrogens is protonated. The molecular topography[15b] of the proton catenate of H was very similar to that of the corresponding Cu(I) catenate. A hydrogen bond analogous to that of GH^+ was observed in the crystal structure of the proton catenate. Due to the insolubility of H in water, the basicity of the phenanthroline of H was examined in $CD_2Cl_2-CD_3CN$. Although reliable measurement of pK_a is not feasible in this solvent, phenanthroline of H was shown to be considerably more basic than the open analogue. The stability of the proton catenate in solution was attributed to charge transfer from the phenoxy ring to the phenanthrolinium acceptor. The stacking interaction between the phenoxy group and phenanthrolinium moiety proposed for proton catenate of H is not present in GH^+. Instead, the stabilization of the phenanthrolinium ion of GH^+ is attributable to hydrogen bond formed with the adjacent phenanthroline as in GH^+.

To provide another piece of evidence for close proximity between the phenanthrolines in $[apoPhen_2]^{PA}$AcPEI, binding of Cu(II) ion to $[apoPhen_2]_{0.56}^{PA}$AcPEI and $[Phen]_{0.81}^{Ran}$AcPEI was examined. The pH dependence (Figure 8) of log K_f for $[apoPhen_2]_{0.56}^{PA}$AcPEI or $[Phen]_{0.81}^{Ran}$AcPEI indicates that Cu(II) ion binds to $[apoPhen_2]_{0.56}^{PA}$AcPEI only when both of the two phenanthrolines of the Cu(II) binding site are unprotonated and to $[Phen]_{0.81}^{Ran}$AcPEI when each phenanthroline moiety is in the basic form. The value (= log K_f°) of log K_f expected when the phenanthrolines of $[apoPhen_2]_{0.56}^{PA}$AcPEI are fully deprotonated is estimated as 17.0.

Effectiveness of cooperation between the two phenanthroline moieties located within a binding site for the Cu(II) ion of $[apoPhen_2]_{0.56}^{PA}$AcPEI may be expressed in terms of effective molarity (EM). The idea of EM has been originally introduced as a measure of efficiency of intramolecular catalysis in comparison with intermolecular catalysis.[19] The concept of EM may be extended to the intramolecular interaction of ligating groups.

Binding of the Cu(II) ion to $[apoPhen_2]_{0.56}^{PA}$AcPEI can be dissected into two processes as illustrated in Scheme 3. The values of log $K_1^\ddagger$ and log $K^\ddagger$ for the Cu(II) complex of B indicated in the scheme have been reported[14] as 5.2 and 11.0 at 25 °C with an ionic strength of 0.1 M.[20] If $K_1^\ddagger$ for Cu(II) binding to B is approximated to be the same as K_{f1}° for Cu(II) binding to the first phenanthroline moiety of $[apoPhen_2]_{0.56}^{PA}$AcPEI,[17] $K_f^\circ/K^\ddagger$ is identical with $K_{f2}^\circ/K_2^\ddagger$. The EM of the second phenanthroline moiety of $[apoPhen_2]_{0.56}^{PA}$AcPEI in the binding to the Cu(II) complex containing one phenanthroline is represented by $K_{f2}^\circ/K_2^\ddagger$. Then, the EM of a phenanthroline toward Cu(II) ion bound to the other phenanthroline in each catenand built on $[apoPhen_2]_{0.56}^{PA}$AcPEI is estimated as 10^6 M (= $K_f^\circ/K^\ddagger$).[21]

The EM measured for $[apoPhen_2]_{0.56}^{PA}$AcPEI may be compared with that for enterobactin, the siderophore with the highest binding constant among the microbial Fe(III) sequestering agents.[22] Enterobactin contains three catechol units. When analyzed according to the method described above by using the formation constant[22b] reported in the literature for the Fe(III) complex of

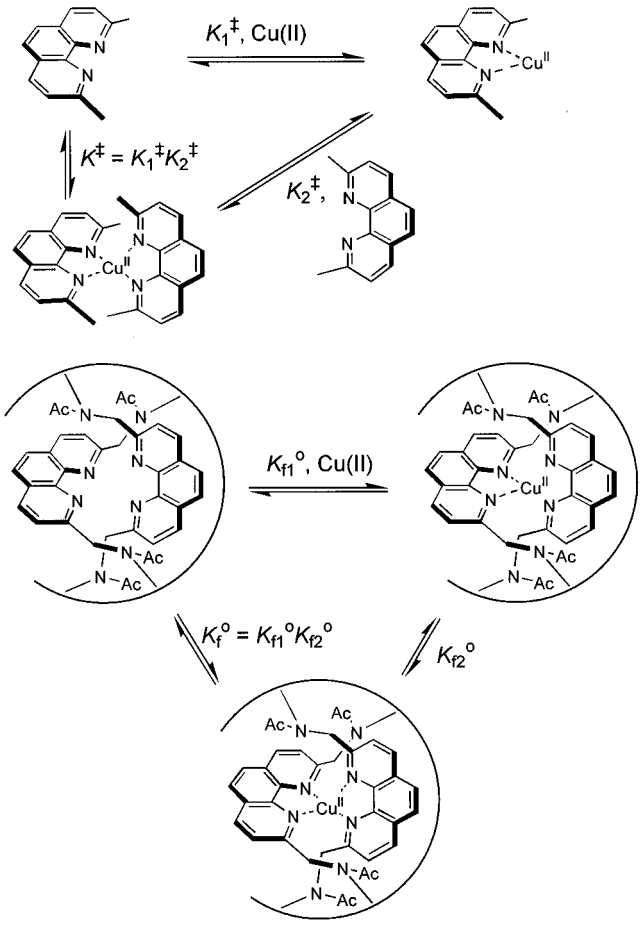
(19) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183.

(20) The value of K_f for $[Phen]_{0.81}^{Ran}$AcPEI (Table 2) is much larger than $K_1^\ddagger$, suggesting that oxygen atoms of acetamide groups or nitrogen atoms of tertiary amino groups as well as the phenanthroline moieties are involved in the binding of Cu(II) ion by $[Phen]_{0.81}^{Ran}$AcPEI.

(21) When the medium is made less polar, the bisphenanthroline complex of Cu(II) would be destabilized whereas phenanthroline ligand would be stabilized. Then, the microenvironment of $[apoPhen_2]_{0.56}^{PA}$AcPEI might reduce the EM value, and the EM value of 10^6 M could underestimate the actual affinity of $[apoPhen_2]_{0.56}^{PA}$AcPEI for Cu(II) ion.

(22) (a) Raymond, K. N. In *Topics in Current Chemistry*; Boschke, F. L., Ed.; Springer Verlag: Berlin, 1984; Vol. 123, p 49. (b) Loomis, L. D.; Raymond, K. N. *Inorg. Chem.* **1991**, *30*, 906.

Scheme 3



enterobactin and that[14] of a reference catechol derivative (2,3-dihydroxy-*N*,*N*-dimethylbenzamide), the EM of a catechol unit toward Fe(III) ion bound to another catechol unit of enterobactin is estimated as 3×10^4 M. Enterobactin contains three catechols units, whereas the Cu(II) binding site of $[apoPhen_2]_{0.56}{}^{PA}$AcPEI consists of only two phenanthrolines. Nevertheless, the EM value observed for $[apoPhen_2]_{0.56}{}^{PA}$AcPEI is extraordinary for an artificial system.

The stabilization of a phenanthrolinium cation by the adjacent phenanthroline is reflected by the large increase in pK_a. The close proximity between the two phenanthrolines contained in a Cu(II) binding site is revealed by the EM value of 10^6 M. These indicate that the geometry of coordination sphere of $Cu(I)A_2$ is effectively conserved[23] during the cross-linkage with PEI followed by removal of Cu(I) ion and acetylation of the primary and secondary amines of the polymer. The effective conservation of the preassembled geometry in $[apoPhen_2]^{PA}$PEI or $[apoPhen_2]^{PA}$AcPEI is due to the highly branched structure of PEI which suppresses the conformational freedom of the two macrocycles. The cross-linkage of organic molecules preassembled by a metal ion with a branched polymer, therefore, can effectively introduce two or more convergent organic functional groups on the synthetic polymer. This methodology can be exploited in the design of many biomimetic functional molecules including artificial enzymes.

Acknowledgment. This work was supported by grants from S.N.U. Daewoo Research Fund and from Korea Science and Engineering Foundation through the Center for Molecular Catalysis at Seoul National University.

Supporting Information Available: UV−vis spectrum of $[apuPhen_2]_{0.56}{}^{PA}$PEI, $[apoPhen_2]_{0.56}{}^{PA}$AcPEI, $[Phen]_{0.81}{}^{Ran}$PEI, and $[Phen]_{0.81}{}^{Ran}$AcPEI measured at 25 °C and various pHs (Figures S1−S4) (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(23) The geometry of the phenanthroline pair in GH^+ or in the Cu(II) complex of G would be somewhat different from that in the corresponding Cu(I) complex.[15a] The high affinity for proton or Cu(II) of G suggests that the phenanthroline pair is conformationally flexible enough to adapt its geometry upon protonation or binding of Cu(II) ion.